AWARD NUMBER: W81XWH-15-1-0160

TITLE:
Cyclin E1 as a Therapeutic Target in Women with High-Grade Serous Ovarian Cancer and Primary Treatment Failure

PRINCIPAL INVESTIGATOR: Professor David Bowtell, PhD

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The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.
A significant number of women with high-grade serous ovarian cancer (HGSC) are intrinsically refractory to standard platinum-based treatment. We have previously shown that amplification of the cyclin E1 gene (CCNE1) in HGSC is associated with primary chemoresistance and poor clinical outcome. Therefore, we hypothesized that cyclin E1 is a key therapeutic target in HGSC, and that generation of a genetically engineered mouse (GEM) model of CCNE1-amplified HGSC will facilitate the development of novel therapeutic strategies. Here, we have generated two mouse strains with Cre-mediated expression of full-length or truncated Ccne1 at the Rosa26 locus. We plan to cross these mice with Pax8-TetOCre-Tp53 mice in order to induce expression of Ccne1 in the fallopian tube epithelium and drive the initiation and development of HGSC.

Mouse models that closely resemble human disease have been powerful platforms for new therapies and understanding resistance mechanisms. Immune checkpoint inhibitors have shown substantial activity in melanoma and lung cancer, and it is now a priority to extend these findings to other solid cancers, including HGSC. The availability of an intact animal model of CCNE1 is likely to be a substantial value in development of immune checkpoint inhibitors and other approaches to targeting CCNE1 amplified tumours.
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1. **INTRODUCTION**

A significant number of women with high-grade serous ovarian cancer (HGSC) are intrinsically refractory to standard platinum-based treatment. We have previously shown that amplification of the cyclin E1 gene (CCNE1) in HGSC is associated with primary chemoresistance and poor clinical outcome. Cyclin E1 complexes with CDK2 to regulate cell cycle G1/S transition. Deregulation of cell cycle control is thought to be a prerequisite for tumour development and several studies have shown accelerated entry into S phase due to constitutive expression of CCNE1. Cyclin E1 is also able to induce chromosome instability by inappropriate initiation of DNA replication and centrosome duplication. We have demonstrated that CCNE1-amplified tumour cells are highly sensitive to knockdown of CCNE1 or CDK2. Our current goal is to further advance novel therapeutic approaches to targeting CCNE1 amplification through the development and characterisation of genetically engineered mouse (GEM) models of Ccne1-amplified HGSC. We also aim to identify and characterise additional genetic events that enhance cyclin E1-mediated transformation in fallopian tube secretory epithelial cells (FTSECs), the likely cell of origin in HGSC, using a high-throughput CRISPR-mediated genome-wide loss of function (LOF) screen. Results generated from this study have significant potential for clinical translation in HGSC, as well as other solid tumours with high frequency of CCNE1 amplification including gastric, breast, and esophageal cancer.

2. **KEYWORDS**

Cyclin E1, CCNE1, amplification, genomic instability, high-grade serous ovarian cancer (HGSC), chemoresistance, mouse models, fallopian tube, homologous recombination (HR)

3. **ACCOMPLISHMENTS**

(A) **Major Goals of the Project**

Site 1: Peter MacCallum Cancer Centre; Melbourne, VIC, Australia (Bowtell)
Site 2: University of Pennsylvania; Philadelphia, PA, USA (Drapkin)
Site 3: Dana-Farber Cancer Institute; Boston, MA, USA (Berhoukim)

<table>
<thead>
<tr>
<th>SPECIFIC AIM 1: GENERATE A TRANSGENIC MOUSE MODEL</th>
<th>Timeline (Months)</th>
<th>Site 1 (PI)</th>
<th>Site 2 (Co-PI)</th>
<th>Site 3 (Co-PI)</th>
<th>% Completed</th>
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<tbody>
<tr>
<td>Major Task 1: Generate transgenic CAG-LSL-Ccne1 mice</td>
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<tr>
<td>Subtask 1: Generate transgenes using the CMV early enhancer/chicken beta-actin (CAG) promoter, followed by a loxP-STOP-loxP cassette to provide conditional expression of full-length and low molecular weight forms of mouse Ccne1</td>
<td></td>
<td></td>
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</table>
## Subtask 2: Rosa26 knock-in- CAG promoter-driven, full length or LMW Ccne1 cDNA will be knocked into the ROSA26 locus by homologous recombination

<table>
<thead>
<tr>
<th>Time</th>
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</thead>
<tbody>
<tr>
<td>3-6</td>
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</tbody>
</table>

## Subtask 3: Southern Blot analysis to determine precise integration via homologous recombination in mouse ES cells. Blastocyst injection of targeted ES into sterile mouse embryos to generate a founder line.

* Outsourced to Ozgene

## Major Task 2: Generate compound mutant mice by intercross and induce tumors

### Subtask 1: Submit documents for local IRB and Animal Ethics review.
- Submission of institution approved animal protocols and related material for DoD’s ACURO approval.
- Receive ACURO approval before initiating animal experiments.

<table>
<thead>
<tr>
<th>Time</th>
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<tbody>
<tr>
<td>1-6</td>
<td>x</td>
<td>x</td>
</tr>
</tbody>
</table>

### Subtask 2: Interbreed breed CAG-LSL-Ccne1 founding mice to expand the colony and obtain homozygous stock.
- Evaluate observed genotype ratios of animals generated for evidence of intrauterine or perinatal mortality and survey tissues from neonatal and adult mice histologically for normal development.

<table>
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<th>Time</th>
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<tr>
<td>12-18</td>
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### Subtask 3: Intercross to Pax8 Cre-deleter mice, also carrying Tp53 (R270H) and Pten^-/- alleles. Confirm genotype.

<table>
<thead>
<tr>
<th>Time</th>
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<tbody>
<tr>
<td>15-18</td>
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</tbody>
</table>

### Subtask 4: Ship compound CAG-LSL-Ccne1, Ccne1; Tp53; Cre deleter, and Ccne1; Tp53; Pten; Cre deleter mice to Drapkin lab and establish stock for investigation of early lesion studies

<table>
<thead>
<tr>
<th>Time</th>
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</table>

### Subtask 5: Administration of doxycycline (0.2mg/ml ad libitum) in the drinking water to induce expression of cyclinE1, mutant Tp53, and Pten deletion
- Monitor animals by ultrasound, body weight, and...
physical examination on a weekly basis for tumor development.
- Ship tumor blocks to Drapkin lab.

**Milestone #1: Established the Ccne1 model**

**Major Task 3: Pathologically and genomically characterize tumours. Compare tumors in different transgenic and mutant backgrounds**

<table>
<thead>
<tr>
<th>Subtask 1: Evaluate Pax8, Stathmin-1, Ccne1, Ki-67, Pten and Tp53 expression compared to controls using immunohistochemistry</th>
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<th>Site 1</th>
<th>Site 2</th>
<th>Site 3</th>
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<table>
<thead>
<tr>
<th>Subtask 2: Evaluate DNA copy number by low coverage (~6X) whole genome sequencing (WGS). Compare copy number profiles to human HGSC with CCNE1 amplification to identify syntenic regions of gain or loss</th>
<th>Months</th>
<th>Site 1</th>
<th>Site 2</th>
<th>Site 3</th>
<th>% Completed</th>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Subtask 3: Characterize the timing and penetrance of tumor development and investigate early lesions</th>
<th>Months</th>
<th>Site 1</th>
<th>Site 2</th>
<th>Site 3</th>
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</table>

**Milestone #2: Characterized tumours and co-authored manuscript.**

**SPECIFIC AIM 2: INTERSECT FUNCTIONAL STUDIES IN FTSECS AND PAN-CANCER TUMOR GENOMIC DATA TO IDENTIFY GENES THAT COOPERATE WITH CCNE1 IN THE TRANSFORMATION OF FTSEC TO GUIDE THE DEVELOPMENT OF COMPOUND CYCLINE1 GEM MODELS.**

**Major Task 4: shRNA screen of FTSEC over expressing CCNE1 performed in Victorian Centre for Functional Genomics (VCFG)**

<table>
<thead>
<tr>
<th>Subtask 1: Transduce cells at an MOI of ~0.3. Select cells by puromycin sensitivity and grow in soft agar. Isolate all the organoids, extract genomic DNA and perform next generation sequencing to identify shRNAs that are statistically abundant and potential drivers of the phenotype.</th>
<th>Months</th>
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<th>Site 2</th>
<th>Site 3</th>
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<table>
<thead>
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<th>Subtask 2: Utilise the VCFG bioinformatics pipeline for analysis of the sequencing data.</th>
<th>Months</th>
<th>Site 1</th>
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</tr>
</tbody>
</table>
(B) Accomplishments Under These Goals

a. Major Activities

We primarily focused on the generation of transgenic CAG-LSL-Ccne1 mice (Major Task 1) during the reporting period.

b. Specific Objectives

In order to complete the generation of transgenic CAG-LSL-Ccne1 mice we had the following objectives:

- Identify a suitable promoter to drive Ccne1 transgene expression in mice
- Design a vector for homology-mediated targeting of Ccne1 to the Rosa26 locus
- Perform successful ESC targeting and blastocyst injection to generate transgenic animals

c. Significant Results or Key Outcomes

Testing of the CAG & UBC promoters in FTSECs. As stated in the proposal, we planned to use the synthetic CAG promoter to drive expression of Ccne1 at the Rosa26 locus as it expressed across a wide range of mouse tissues and has been used extensively for transgene expression in mice. We also wanted to compare it to the human UBC promoter, which is also commonly used for transgene expression. As neither promoter had been targeted to FTSEC, we could not predict which would be more appropriate for our studies. Therefore as an initial guide, we generated GFP constructs with each promoter and transfected these into FTSEC lines to monitor expression. Under optimal transfection conditions, we found that the CAG promoter was able to drive strong GFP expression in 35.9% of transfected FTSECs versus the UBC promoter, which only stimulated GFP expression in 12.7% of cells (Figure 1). These results indicated that the CAG promoter was the most suitable for expression of Ccne1 in mice. Subsequently, Ozgene approached us about pursuing the comparison of the CAG and UBC promoters in vivo, and agreed to generate an additional UBC-LSL-Ccne1 mouse strain for direct comparison with the CAG-LSL-Ccne1 at no cost.
Successful generation of UBC-LSL-CCNE1 and CAG-LSL-LMW_CCnec1 transgenic mice. Generation of the Ccne1 transgenic mice was outsourced to Ozgene. Targeting vectors (Figure 2) were designed and produced to our specifications and included: the CAG or UBC promoter, a LoxP-STOP-LoxP (LSL) cassette, a neomycin selectable marker, and the coding sequence (cDNA) for either full-length or a low molecular weight (LMW) isoform of Ccne1 (Appendix A). Ozgene was able to successfully integrate UBC-LSL-Ccne1 and CAG-LSL-LMW_Ccne1 into the Rosa26 locus in ES cells and generate homozygous stocks of mice (Figure 3 & Figure 4). These mice were subsequently shipped to the Peter MacCallum Animal Facility, where we have been able to successfully genotype, expand, and maintain colonies (Figure 5). Furthermore, to facilitate the generation of compound mutant mice, both the Bowtell and Drapkin laboratories have submitted animal ethics protocols and received approval from their respective Animal Ethics Committees (Appendix B & Appendix C).

Multi-copy CAG-LSL-Ccne1 transgenic mice. Ozgene also reported successful generation of CAG-LSL-Ccne1 targeted ES cells and subsequent germline transmission in mice. However, in the course of further characterisation of the mice it became apparent that there are multiple integrants in the locus, perhaps up to 10 copies. We have obtained these mice and continue to characterise them while Ozgene has returned to generating single copy precise intergrants of the CAG-LSL-Ccne1 transgene. It may be that the additional copies will provide enhanced expression that is serendipitously useful or be transcriptionally leaky and not appropriate for our studies. Will characterise the single and multiple copy animals until we have a clear outcome.

Optimization of soft agar growth conditions for FTSECs for the genome-wide screen (Aim 2). We have optimized conditions for soft agar growth of FTSECs in 6-well tissue culture dishes to facilitate positive selection in the genome-wide loss-of-function screen. We are currently working on establishing FTSECs with stably expression of Cas9.

FIGURES:

![Figure 1. CAG and UBC driven expression of GFP in FTSECs. FACs analysis for GFP expression in FTSECs optimally transfected with vectors containing either the CAG or UBC promoter and a GFP reporter protein.](image)
**Figure 2.** Targeting vectors used to facilitate insertion of Ccne1 at the Rosa26 locus. (A) The CAG promoter vector used to generate mice expressing full length or truncated Ccne1. (B) The UBC promoter used to generate mice expressing full length Ccne1. Note the presence of a loxP-STOP-loxP cassette facilitating conditional expression of Ccne1 via Cre recombinase.

**Figure 3.** Successful ES cell targeting and germline transmission of CAG-LSL-LMW_Ccne1. South blot screening of: (A) targeted ES cells identified positive clones as indicated by the black arrows; (B) chimera mice identified successful germline transmission as indicated by *; (C) heterozygous founder mating identified homozygous offspring as indicated by #.

**Expected sizes are:**

- Wild type = 11.5kb
- Transgene = 9.2kb
Figure 4. Successful ES cell targeting and germline transmission of **UBC-LSL-Ccne1**. South blot screening of: (A) targeted ES cells identified positive clones as indicated by the black arrows; (B) chimera mice identified successful germline transmission as indicated by *; (C) heterozygous founder mating identified homozygous offspring as indicated by #.

Figure 5. **Genotyping PCR.** Successful breeding of homozygous **UBC-LSL-Ccne1** and **CAG-LSL-LMW-Ccne1** mice at the Peter MacCallum Animal Facility.
d. Other Achievements

We identified AKT inhibition as a potential therapeutic combination with the CDK2 inhibitor dinaciclib in CCNE1 amplified HGSC cell lines. The results of this study are shown in the attached manuscript (Appendix D: Au-Yeung et al, Clinical Cancer Research, 2016, in press) and are briefly described below.

We showed that CDK2 is a highly selective target for CCNE1 amplified high-grade serous ovarian cancer (HGSC), using siRNA and conditional shRNA systems in vitro and in vivo. However, we found that dinaciclib, a small molecule CDK inhibitor, did not trigger amplicon dependent sensitivity in a panel of HGSC cell lines. This is likely due to the non-specific nature of small molecule CDK inhibitors such as dinaciclib, which although it is a potent inhibitor of CDK2, also inhibits CDK1, 5, 9 and 12. In order to improve on the efficacy of dinaciclib, we conducted a high throughput compound screen of over 4000 known drugs and targeted agents to identify selective synergistic combinations. We also aimed to identify combinations that may overcome drug resistance by including a CDK-inhibitor resistant cell line previously derived from a sensitive, CCNE1-amplified cell line. We obtained a number of hits, and focused particularly on a combination of dinaciclib and AKT inhibitors that were synergistic in CCNE1-amplified HGSC. We orthogonally validated the interaction between CCNE1 and AKT, both in genomic data from TCGA and functionally in fallopian tube secretory cells, the target of HGSC transformation. In summary, our findings support CDK2 as a specific target for CCNE1 amplified HGSC, and identify a combination of dinaciclib and AKT inhibitors that may inform the design of a rational clinical trial targeting an important subset of HGSC.

(C) Opportunities for Training and Professional Development

Nothing to Report

(D) Dissemination of Results to Communities of Interest

Nothing to Report

(E) Plans for Next Reporting Period to Accomplish the Goals

Our overall goal is to develop a GEM model of Ccne1-amplified HGSC to better understand disease initiation and to facilitate the development of novel therapeutic strategies. To this end, over the next year, we will finish generating the CAG-LSL-Ccne1 mouse strain and begin generating compound mutant mice by intercrossing the Ccne1 mouse strains with Pax8-TetO-Cre-Tp53 mice (as outlined in the proposal). Once the compound mutant mice are established, we plan to induce Ccne1 expression and Tp53 deletion via doxycycline administration and systematically monitor mice for tumour development. The Drapkin and Bowtell laboratories will pathologically and genomically characterize any tumours that develop, respectively. Finally, we will finish optimization of conditions (transduction and soft agar growth) and carry out the high-throughput CRISPR-mediated gene knockout screen in FTSECs. Results from the screen will identify tumor suppressor genes that cooperate with CCNE1 in the transformation of FTSECs, and guide the selection of alternative genes to develop additional compound GEM models using the CAG-LSL-Ccne1-Tp53 strains.

4. IMPACT

(A) Impact on the Development of the Principal Discipline(s) of the Project
Nothing to Report

(B) Impact on Other Disciplines

Nothing to Report

(C) Impact on Technology Transfer

Nothing to Report

(D) Impact on Society Beyond Science and Technology

Nothing to Report

5. CHANGES/PROBLEMS

(A) Changes in Approach and Reason for Change

Selection of a CRISPR-Cas9 approach for the loss-of-function screen (Aim 2.1). At the time of the proposal submission, CRISPR-Cas9 was just emerging as a powerful tool for manipulating genomes, including genome-wide loss-of-function (LOF) screens. We had planned to perform an shRNA positive selection screen to identify tumour suppressors that cooperate with CCNE1 in the transformation of FTSECs, however we indicated that we were open to adopting a CRISPR-Cas9 approach as the methodology evolved. Recent publications have compared CRISPR-Cas9 to shRNA-based genome-wide LOF screens in human cancer cell lines and found CRISPR-Cas9 to be superior in identifying essential/lethal genes, which suggests that complete gene inactivation may be necessary to identify cellular dependencies (Munoz et al Cancer Discovery 2016; Aguirre et al Cancer Discovery 2016). Therefore, we have elected to use a CRISPR-Cas9 approach in our genome-wide LOF screen. This screen will be performed in the Victorian Centre for Functional Genomics (VCFG), located at the Peter MacCallum Cancer Centre as previously described in the proposal. The overall workflow for the screen will remain as described for the shRNA screen including soft agar growth selection and next generation sequencing to identify guide RNAs (gRNAs) that are statistically abundant and potential drivers of the phenotype. The VCFG have obtained, expanded, and sequenced the human CRISPR Brunello lentiviral pooled library (two-vector system), which was designed using optimized metrics and consists of four gRNAs per gene for a total of 76,441 gRNAs (Doench et al Nat Biotechnol. 2016). The switch to a CRISPR-based approach results in a slight delay as the VCFG have obtained the libraries and developed virus. During this time we have optimized conditions for soft agar growth of FTSECs, and are currently generating the required FTSEC lines stably expressing Cas9. Since any intercross into mice of additional mutant alleles revealed by the CRISPR screen requires prior characterisation of the GEM mouse model, the delay in initiating the screen will not impact on the overall timeline for the study.

Deprioritization of Pten deletion from the breeding scheme. As stated in the proposal Aim 2.2, we plan to filter hits identified in the CRISPR-mediated LOF screen (and other previously performed cyclin E1 gain-of-function and LOF screens) to identify the most relevant genes to assess in mice. As an initial filter, we utilized pan-cancer data from TCGA and identified genes that are frequently altered by somatic genetic events (amplification or deletion), and for which these events are positively correlated with CCNE1 amplification, suggesting cooperativity. Our preliminary results indicate that PTEN deletion does not significantly co-occur with CCNE1
amplification in human cancers (Table 1). Although loss of Pten significantly reduced tumour latency in PI Drapkin’s BRCA1/2 model we have deprioritized the crossing of CAG-LSL-Ccne1-Tp53 mice with Pten−/− mice, as it would not reflect the biology of human HGSC. It is quite possible that the combination of Ccne1 over expression and Tp53 deletion will be sufficient for generating HGSC in the mouse. However, in parallel, we will also consider other mutations/deletions identified in our loss-of-function screen, which may be introduced into the CAG-LSL-Ccne1-Tp53 GEM model to enhance in vivo transformation.

<table>
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<th>Correlative p-value</th>
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<td>IL17RA, POTECH, CCT8L2, XKR3, CECR7</td>
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<td>2</td>
<td>amp_ERBB2</td>
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<td>ERBB2</td>
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<td>del_KMT2C</td>
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<td>KMT2C</td>
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<td>ENSA, GOLPH3L</td>
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<td>5</td>
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<td>FLOT2, ERAL2, FAM222B, PHF12, SEZ6</td>
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<tr>
<td>106</td>
<td>del_Pten</td>
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<td>PTEN</td>
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Table 1. Genetic Alterations That Correlate with CCNE1 Amplification. GISTIC peaks (from the "2015-06-01 stdddata_2015_04_02 arm-level peel-off" analysis at http://www.broadinstitute.org/tcga) with the p-values for correlation of the peak event with amplification of CCNE1 in the pan-cancer TCGA dataset.

(B) Actual or Anticipated Problems or Delays and Actions or Plans to Resolve Them

Relocation of Peter MacCallum Cancer Centre. In June 2016, Peter MacCallum Cancer Centre relocated to the new Victorian Comprehensive Cancer Centre building in the Parkville precinct of Melbourne. This relocation resulted in the Peter MacCallum Animal Facility being unable to accept external mouse importations from approximately April to August 2016, which impacted the shipment of the Ccne1 mouse strains from Ozgene. To keep the project on track, we contracted Ozgene to interbreed the UBC-LSL-Ccne1 and the CAG-LSL-LMW_Ccne1 founding mice to expand the colonies and obtain homozygous stocks. We received homozygous breeding pairs for both strains in mid-August 2016, and have successfully continued to expand these colonies.

Relocation of the Drapkin Laboratory. During Year 1 of the grant, PI Drapkin accepted a new position at the University of Pennsylvania that required the relocation of his laboratory to Philadelphia, Pennsylvania. Due to the necessary shipment and reestablishment of the Drapkin laboratory mouse strains at the new animal facility, there has been a delay in receiving the Pax8-TetOCre-Tp53 mice required for interbreeding with the Ccne1 mice. We anticipate shipment of three breeding pairs of Pax8-TetOCre-Tp53 mice to Peter MacCallum Cancer Centre in February 2017. Additionally, we have identified a laboratory in Australia that has an alternative Pax8-TetOCre mouse strain that can be imported to Peter MacCallum Cancer Centre if the delay in shipment from the Drapkin laboratory is longer than expected.

Multiple Integrations of Ccne1 in CAG-LSL-Ccne1 Mice. In May 2016, we were informed by Ozgene that in the process of reconfirming integration of Ccne1 in the germline offspring, they identified that the CAG-LSL-Ccne1 mice had multiple integrations of the vector at the Rosa26 locus. Unfortunately, frozen stocks of targeted CAG-LSL-Ccne1 mouse ES cells were not viable, and Ozgene had to begin de novo derivation of the strain. As of this report, Ozgene has achieved successful single integration and germline transmission of CAG-LSL-Ccne1, with heterozygous mice anticipated for shipment in late-January 2017. Although it will be important to have strain with single integration of Ccne1, we believe that CAG-LSL-Ccne1 mice with multiple integrations (CAG-LSL-Ccne1_multi) may also prove useful given that patients with HGSC can...
have more than eight copies of CCNE1. Therefore, we have elected to maintain the CAG-LSL-Ccne1 multi mouse strain (provided by Ozgene at no cost) and characterize it in parallel with the other established mouse strains as an interim measure.

(C) Changes That Had a Significant Impact on Expenditures

Nothing to Report

(D) Significant Changes in Use or Care of Human Subjects, Vertebrate Animals, Biohazards, and/or Select Agents

In regard to animal ethics protocol titled “Genetically Engineered Mouse Models of Ovarian Cancer”, IACUC protocol E561, we have submitted and received approval from the Peter MacCallum Animal Experimentation Ethics Committee to add an additional investigator (Dr. Jessica Beach). The amendment was approved 21 April 2016 and these changes were reported via email to ACURO on 1 September 2016.

6. PRODUCTS

(A) Journal Publications


Acknowledgement of federal support: Yes

(B) Other Products—Research Material

We have generated two of three planned GEM strains with mouse cyclin E1 (Ccne1; full length or truncated) expressed under the control of the CAG or human UBC promoter at the Rosa26 locus. Furthermore, our targeting construct contained a loxP-STOP-loxP cassette allowing for tissue-specific, Cre-mediated excision of the cassette and conditional expression of Ccne1. These mouse strains may be beneficial in the generation of GEM models of other CCNE1-amplified cancers, such as gastric cancer.

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<td>C57Bl6-Gt(ROSA)26Sor^tm1(LOSL-CAG-Ccne1)Ozg</td>
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<tr>
<td>C57Bl6-Gt(ROSA)26Sor^tm1(LOSL-UBC-Ccne1)Ozg</td>
<td>Established</td>
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<tr>
<td>C57Bl6-Gt(ROSA)26Sor^tm1(LOSL-CAG-LMWCcne1)Ozg</td>
<td>Established</td>
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7. PARTICIPANTS

(A) Individuals who have worked on this project

The following individuals have contributed to the project:
Dr. Dariush Etemadmoghdam was named under other personnel as the postdoctoral fellow on this project at Peter MacCallum Cancer Centre, however Dr. Etemadmoghdam has taken on a new role that means he no longer has the required time to commit to the project. In his place, we plan to deploy Dr. Jessica Beach, who also has the required and appropriate experience to be the postdoctoral fellow on this project (see below). As this role is not due to commence until year 2 of this project (1 October 2016) we anticipate that this personnel change will have no impact on the progress of the project or the budget.

Dr. Beach will coordinate all aspects of the study being performed at Peter MacCallum Cancer Centre including animal breeding, animal model characterisation, and the high-throughput gene knockout screen.

(B) Changes in Active Other Support of the PI or Key Personnel

David Bowtell (PI)

Pending to Active Research Support:

APP1092856 (Bowtell) 2016 – 2020
NHMRC Program Grant Improving Outcomes for Women with Epithelial Ovarian Cancer Role: Principal Investigator (40% effort) Awarded: $374,632 AUD (annual direct costs)

OC150563 (Pike/Pearce) 2016 – 2019
US Department of Defense Ovarian Cancer Research Program Multidisciplinary Ovarian Cancer Outcomes Group (MOCOG) Role: Key Personnel (5% effort Year 1, 2, 3; 4% effort Year 4) Awarded: $216,418 (direct costs)
APP 14/TPG/1-15 (deFazio) 2015 – 2019
Cancer Institute NSW Translational Program Grant
INOVATe - Individualised Ovarian Cancer Treatment Through Integration of Genomic Pathology into Multidisciplinary Care
Role: Chief Investigator E (5% effort)

Active to Completed Research Support:

APP1044447 (Bowtell) 2013 – 2015
NHMRC Project Grant
Novel Therapeutic Approaches to Ovarian Clear Cell Cancer
Role: Chief Investigator A

Ronny Drapkin (Co-PI)

Pending to Active Research Support:

No Number (Drapkin) 2013 – 2017
Tina Brozman Foundation
Ex vivo culture of fallopian tube epithelium for proteomic detection of biomarkers in ovarian cancer
Role: Principal Investigator (5% effort)
Awarded: $45,455 (annual direct costs)

No Number (Drapkin) 2016 – 2016
Tina Brozman Foundation
Tina Brozman Ovarian Cancer Consortium (TBOCC)
Role: Principal Investigator (5% effort)
Awarded: $33,174 (annual direct costs)

Active to Completed Research Support:

R21 CA156021 (Drapkin) 2013 – 2015
NIH/NCI
Elafin as a biomarker in serous ovarian cancers and basal-like breast tumors
Role: Principal Investigator (15% effort)
Awarded: $57,925 (annual direct costs)

Rameen Berhoukim (Co-PI)

Pending to Active Research Support:

5 R01 CA188228-02 (Beroukhim, Ligon) 2015 – 2020
NIH Project Grant
Genetic Evolution of Glioblastoma During Radiation and Temozolomide Therapy
Role: Principal Investigator (20% effort)
Awarded: $448,351 (annual direct cost)

No Number (Beroukhim) 2015 – 2016
Ian’s Friend’s Foundation
Identifying Therapeutic Targets of MYB-QK1 Fusions in Pediatric Low-Grade Glioma  
Role: Key Personnel (1% effort)  
Awarded: $100,000 (direct costs)

No Number (Beroukhim)  
Cure Starts Now  
Characterizing resistance mechanisms to radiation and adjuvant therapy in DIPG  
Role: Key Personnel (1% effort)  
Awarded: $90,909 (direct costs)

Footridge Award (Lindquist, Santagata, Beroukhim)  
KI-DF/HCC Bridge Program  
Modulating the heat-shock machinery to limit genetic heterogeneity and evolution of highly malignant cancers  
Role: Key Personnel (2% effort)  
Awarded: $41,288 (Beroukhim portion, direct costs)

Innovation Grant (Beroukhim)  
Alex’s Lemonade Stand Foundation  
Characterizing resistance mechanisms to BET-bromodomain inhibition of MYC-amplified medulloblastoma  
Role: Principal Investigator (12.5% effort)  
Awarded: $125,000 (annual direct cost)

No Number (Beroukhim)  
Broad Institute SPARC (Scientific Projects to Accelerate Research & Collaboration)  
EvoSeq: A molecular time machine to study evolution  
Role: Key Personnel (2% effort)  
Awarded: $185,000

No Number (Beroukhim)  
St. Baldrick’s Foundation  
Intratumoral heterogeneity of resistance drivers in DIPG  
Role: Key Personnel (1% effort)  
Awarded: $100,000 (direct costs)

Pediatric Brain Tumor Foundation  
BRD4 as a therapeutic target in medulloblastoma  
Role: Principal Investigator (5% effort)  
Awarded $100,836 (annual direct costs)

U24 CA210978-01 (Beroukhim, Cherniack)  
NIH Resource-Related Research Projects  
Center for the comprehensive analysis of somatic copy-number alterations in cancer  
Role: Principal Investigator (20% effort)  
Awarded: $412,500 (annual direct costs)

(C) Other Partner Organizations

Nothing to Report
8. SPECIAL REPORTING REQUIREMENTS

None

9. APPENDIX

Appendix A: Ccne1 Sequences Used for Targeting
Appendix B: E561 Animal Ethics Approval, Peter MacCallum Cancer Centre (Bowtell)
Appendix C: 806138 Animal Ethics Approval, University of Pennsylvania (Drapkin)
APPENDIX A: Ccne1 Sequences Used for Targeting

CAG-LSL-Ccne1 (1615_Truman)

>Mouse_CCNE1_FullLength_CDS
ATGCCAAGGGAGAGAGCTCGACGGACCACAGCAACATGAAAGAAGAAGGTGGCTCCGACCTTTCAGTCCGCTCCAGAAAAAGGACCAATCTGCCAGCTGGTTTGGTCAAGACCAACAGATGAAGAAATTGCCAAGATTGACAAGACTGTGAAAA
GCGAGGATACGCTGGCTCATCTCCTCTGTGACCCGAGATGAGAGAGATCTCTCTGGCTATGTTGGAAGTGGTGCC
GAAGTCTATAAGTCCACACAGAGACTCTTCTACTGGACACAGACTCTTCTTGGATGTTACATGGCAATACAAATA
TACTATAAAACACCTTTCACGTCTATTGGGATTCCTACGCCCTCAAAAATCTGAGAAATGGAAAACTCAGTGAGATC
GCTTCCTAGGTAGTGGCTCTTGAATTTCTCCTATGGTGCCTGGCTCTTTCATCCCCACCCCTAACAAAGAAGAGGACA
ATGAGCTTGAATACCCCAGGACTGCATTTCAGCCTCGGAAAATCAGACCACCCAGAGCCTCCCCACCTTCCCGTCTT
GAATTGGGGCAATAGAGAAGAGGTTTGGAGGATCATGTTAAACAAAGAAAAGACTTACCTGAGAGATGACATTTCTG
CTGCAGCGTCATCCTCTCCTGCAGGCGAGGATGAGAGCAGTTCTTCTGGATTGGCTAATGGAGGTGTGCAGAAGTCTA
ATAAGCTCCACAGAGAGACGTTCTACTTGGCACAGGACTTCTTTGATCGTTACATGGCATCACAACATAATATCATA
AAAACACTTTTACAGCTTATTGGGATTTCAGCCTTATTTATTGCTTCAAAACTTGAGGAAATCTACCCTCCAAAGTTG
ACCAGTTTGCTTATGTTACAGATGGCGCTTGCTCCGGGGATGAAATTCTTACCATGGAATTGATGATGATGAAGGCCCT
TAAGTGGCGTCTAAGCCCTCCTGACCATTGTGTCCTGGCTGAATGTCTATGTCCAAGTGGCCTATGTCAACGACACGGG
TGCAGATCGCTGGCTCTCCGGGATGTTGGCTGCTTAGAATTTCCTTATGGTGTCCTCGCTGCTTCTGCTTTGTATCAT
TTCTCACTGGAGTTGATGCAGAAGGTCTCAGGTTATCAGTGGTGCGACATAGAGAAGTGTGTCAAATGGATGGTTCCG
TCGCCATGGTTATCCGGGAGATGGGAAGTTCCAAGCTCAAGCACTTCCGGGGAGTCCCCATGGAAGACTCCCACAAC
ATCCAGACCCACACCAACAGCTTGGATTTGCTGGACAAAGCCCAAGCAAAGAAAGCCATATTGTCAGAACAGAATAGG
ATTTCTCCTCCTCCGAGTGTGGTCCTGACACCCCCACCCAGCAGTAAGAAGCAGAGCAGCGAGCAGGAGACAGAATGA

>Mouse_CCNE1_FullLength_Protein
MPRERDSTDHSMKEEGGDSLVRSSRKRANVAFLQPDPDEEIAIKIDTVKSEDSSQPWDDNASCVPDCSFIPTPNK
EEDNELEPYRAPQPRKPRASPLPVNLWGNEREVWRVRLLNKLLYLRDEHFLRQHRPLQQRAVMRLDLWLMVCE
EVYKLRHETFYLAQDFDRYMAQSNHINIKTLQLIGISALFASKEEYIPKHLQFAYVTGDACSGDEILTMLELMM
MKALKWSLPITSLVSLLNYVTQVAYVNDTEGILMPQYPQVFQVIAELLDLCLVDLCLEFLQVPYGLAALASYHFSSL
ELMQRVSQVYQCDIEKCVKWNMFVPAMVREMSSKLKHFHRGVMEDSHNIQTHNTSLDLLDKAQKAILSEQNRIS
PPSVVLTPPPSSKQKSEQUETE

CAG-LSL-Ccne1-Truncated (1616_Enoch)

>Mouse_CCNE1_T1_Truncated_CDS
ATGGACCCAGATGGAATAATTGCCAAGATTGGAACACACTCTGGAAAGAGGAGAAGATCAGCCCCTTGGATGAA
TTCCAGATGCTGAGCCCTCCTTTTCATCCCTCCCCTCCTCACCCTAACAAAGAAGAGGACATGGAATTGAGAGAGAT
CTCTGCTTGCATGCTGGCTCTTGAATTTCTCCTATGGTGCCTGGCTCTTTCATCCCCACCCCTAACAAAGAAGAGGACA
ATGAGCTTGAATACCCCAGGACTGCATTTCAGCCTCGGAAAATCAGACCACCCAGAGCCTCCCCACCTTCCCGTCTT
GAATTGGGGCAATAGAGAAGAGGTTTGGAGGATCATGTTAAACAAAGAAAAGACTTACCTGAGAGATGACATTTCTG
CTGCAGCGTCATCCTCTCCTGCAGGCGAGGATGAGAGCAGTTCTTCTGGATTGGCTAATGGAGGTGTGCAGAAGTCTA
ATAAGCTCCACAGAGAGACGTTCTACTTGGCACAGGACTTCTTTGATCGTTACATGGCATCACAACATAATATCATA
AAAACACTTTTACAGCTTATTGGGATTTCAGCCTTATTTATTGCTTCAAAACTTGAGGAAATCTACCCTCCAAAGTTG
ACCAGTTTGCTTATGTTACAGATGGCGCTTGCTCCGGGGATGAAATTCTTACCATGGAATTGATGATGATGAAGGCCCT
TAAGTGGCGTCTAAGCCCTCCTGACCATTGTGTCCTGGCTGAATGTCTATGTCCAAGTGGCCTATGTCAACGACACGGG
TGCAGATCGCTGGCTCTCCGGGATGTTGGCTGCTTAGAATTTCCTTATGGTGTCCTCGCTGCTTCTGCTTTGTATCAT
TTCTCACTGGAGTTGATGCAGAAGGTCTCAGGTTATCAGTGGTGCGACATAGAGAAGTGTGTCAAATGGATGGTTCCG
TCGCCATGGTTATCCGGGAGATGGGAAGTTCCAAGCTCAAGCACTTCCGGGGAGTCCCCATGGAAGACTCCCACAAC
ATCCAGACCCACACCAACAGCTTGGATTTGCTGGACAAAGCCCAAGCAAAGAAAGCCATATTGTCAGAACAGAATAGG
ATTTCTCCTCCTCCGAGTGTGGTCCTGACACCCCCACCCAGCAGTAAGAAGCAGAGCAGCGAGCAGGAGACAGAATGA

>Mouse_CCNE1_T1_Truncated_Protein
MPRERDSTDHSMKEEGGDSLVRSSRKRANVAFLQPDPDEEIAIKIDTVKSEDSSQPWDDNASCVPDCSFIPTPNK
EEDNELEPYRAPQPRKPRASPLPVNLWGNEREVWRVRLLNKLLYLRDEHFLRQHRPLQQRAVMRLDLWLMVCE
EVYKLRHETFYLAQDFDRYMAQSNHINIKTLQLIGISALFASKEEYIPKHLQFAYVTGDACSGDEILTMLELMM
MKALKWSLPITSLVSLLNYVTQVAYVNDTEGILMPQYPQVFQVIAELLDLCLVDLCLEFLQVPYGLAALASYHFSSL
ELMQRVSQVYQCDIEKCVKWNMFVPAMVREMSSKLKHFHRGVMEDSHNIQTHNTSLDLLDKAQKAILSEQNRIS
PPSVVLTPPPSSKQKSEQUETE
APPENDIX B: E561 Animal Ethics Approval, Peter MacCallum Cancer Centre (Bowtell)

PETER MACCALLUM CANCER CENTRE
ANIMAL EXPERIMENTATION ETHICS COMMITTEE (AEEC)

Application For Approval To Use Animals In A Research Project

| Date application received: 23/11/15 |
| Resubmitted: 15/02/16 |

Office Use Only

Project Title: Genetically engineered mouse models of ovarian cancer

AEEC Register Number
AEEC Permit Number: E 5 6 1

DECLARATION BY AEEC CHAIRMAN

I certify that this project has been considered and approved by the Peter MacCallum Cancer Centre AEEC Chair on the 7th March 2016 and ratified by the full committee on the 26th May 2016.

The period of approval for this project is 07/03/16 to 06/03/19

AEEC Chairman Name: A/Prof. Phillip Darcy
AEEC Chairman Signature: 
Date: 31/05/16

CONDITIONS OF APPROVAL

All matters pertaining to the conduct of the approved project are to be reported to the AEEC, which maintains oversight in accordance with licence conditions for the Licence SPPL078.

Any variation proposed to the project, and the reasons for that change, must be submitted to the AEEC for approval and must not be implemented until approval is granted.

A record of details of any animals used in the project must be retained.

The project should only be conducted in approved premises nominated on the Bureau of Animal Welfare Scientific Licence SPPL078.

The AEEC must also be notified in writing of:

- Any changes to approved investigators
- Any unexpected incidents or complications that result in deaths, euthanasia or pain and suffering for the animals used in the project. Details of the steps taken to deal with adverse incidents must be included in the notification.

OTHER CONDITIONS:

This approval is subject to the following special conditions:
APPENDIX C: 806138 Animal Ethics Approval, University of Pennsylvania (Drapkin)

UNIVERSITY OF PENNSYLVANIA
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)
(Animal Welfare Assurance # A3079-01)

RONNY DRAPKIN
4333 - OB-Obstetrics and Gynecology
421 Curie Blvd
BRB II/III Suite 1215
Philadelphia, PA 19104-6160

10-Nov-2016

PRINCIPAL INVESTIGATOR : RONNY DRAPKIN
PROTOCOL TITLE : Using Genetically Engineered Mice for the Study High Grade Serous Ovarian Cancer
PROTOCOL # : 806138
3-YEAR APPROVAL PERIOD : 05-Nov-2016 – 05-Nov-2019

Dear DR. DRAPKIN:

The above referenced protocol was reviewed and approved by the Institutional Animal Care and Use Committee on 05-Nov-2016. Protocols are valid for three years from the date of approval. However, please note that protocols with USDA-covered species will require annual continuing reviews. This study will be due for its next review on or before 05-Nov-2019. Please log into the ARIES electronic protocol system (https://aries.apps.upenn.edu/laProtocol.jsp?fast2.jsp) on a routine basis to check the status of your protocol, and be cognizant of reminder notifications that will be sent out when new submissions are required.

Please note that the principal investigator should contact ULAR to verify animal housing availability and to coordinate activities for any special animal study needs (including special housing) or equipment requirements if this was not done during the planning phases of the protocol. IACUC protocol approval DOES NOT guarantee the availability of required resources for animal work.

Please take note of the following information:

**Personnel Training:** It is the responsibility of the Principal Investigator to ensure that all persons have completed all necessary training prior to participating in the research described in this protocol.

**Submissions:** Please note that all future submissions related to this protocol must be submitted within ARIES. No paper submissions will be accepted.

**Amendments:** If you wish to change any aspect of this study, such as personnel, sponsors, hazardous materials, drugs, or procedures, please submit an Amendment to the protocol within ARIES. The new changes cannot be initiated until IACUC approval has been given.

**Completion of Study:** Please notify the Office of Animal Welfare once the research has been completed so the protocol can be terminated. This will prevent you from receiving unnecessary reminder notifications for renewal submissions.

If you have any questions, please contact our office as indicated above. Thank you for your cooperation with the Committee.

Sincerely,

[Signature]

Digitally signed by Gregory R. Reinhard
Date: 2016.11.14 09:09:32 -05'00'

Gregory R. Reinhard, MBA, DVM, DACLAM
Director, Office of Animal Welfare
Selective Targeting of Cyclin E1-Amplified High-Grade Serous Ovarian Cancer by Cyclin-Dependent Kinase 2 and AKT Inhibition

Abstract

Purpose: Cyclin E1 (CCNE1) amplification is associated with primary treatment resistance and poor outcome in high-grade serous ovarian cancer (HGSC). Here, we explore approaches to target CCNE1-amplified cancers and potential strategies to overcome resistance to targeted agents.

Experimental Design: To examine dependency on CDK2 in CCNE1-amplified HGSC, we utilized siRNA and conditional shRNA gene suppression, and chemical inhibition using dinaciclib, a small-molecule CDK2 inhibitor. High-throughput compound screening was used to identify selective synergistic drug combinations, as well as combinations that may overcome drug resistance. An observed relationship between CCNE1 and the AKT pathway was further explored in genomic data from primary tumors, and functional studies in fallopian tube secretory cells.

Results: We validate CDK2 as a therapeutic target by demonstrating selective sensitivity to gene suppression. However, we found that dinaciclib did not trigger amplicon-dependent sensitivity in a panel of HGSC cell lines. A high-throughput compound screen identified synergistic combinations in CCNE1-amplified HGSC, including dinaciclib and AKT inhibitors. Analysis of genomic data from TCGA demonstrated coamplification of CCNE1 and AKT2. Overexpression of Cyclin E1 and AKT isoforms, in addition to mutant TP53, imparted malignant characteristics in untransformed fallopian tube secretory cells, the dominant site of origin of HGSC.

Conclusions: These findings suggest a specific dependency of CCNE1-amplified tumors for AKT activity, and point to a novel combination of dinaciclib and AKT inhibitors that may selectively target patients with CCNE1-amplified HGSC. Clin Cancer Res 1–15.

Introduction

Targeted therapies have changed the management of many cancers types, resulting in significant improvements in clinical response rates and survival (1). However, the antiangiogenic mAb bevacizumab (2, 3) and the PARP inhibitor olaparib (4, 5) have entered care in high-grade serous ovarian cancer (HGSC) recently, the development of targeted therapy to this disease has been relatively slow.

HGSCs are characterized by ubiquitous TP53 mutations, genomic instability, and widespread copy number alterations, with relatively infrequent somatic point mutations of driver genes (6, 7). Structural alteration also contributes to loss of tumor suppressors such as RB1 and NF1 by gene breakage (8). Defects in the homologous recombination repair (HR) pathway are present in approximately 50% of HGSCs, primarily associated with germ-line and somatic mutations in BRCA1, BRCA2, and associated proteins (7). HR deficiency impacts platinum sensitivity in HGSC, and provides the basis for the use of PARP inhibitors that target compensatory DNA repair pathways (4, 9). Of HGSC with intact HR, amplification of CCNE1, which encodes the cell-cycle regulator cyclin E1, is the best characterized driver. CCNE1 amplification or gain occurs in 20% of all HGSC tumors and is associated with primary treatment resistance and reduced overall survival in HGSC (10, 11). Patients whose tumors have CCNE1...
Translational Relevance

High-grade serous ovarian cancer (HGSC) patients with Cyclin E1 (CCNE1) amplification represent a group with high unmet clinical need. Novel therapies are needed to improve outcomes in these patients, given that CCNE1-amplified tumors are unlikely to respond to chemotherapy or PARP inhibitors, and are associated with poor overall survival. Here, we validate CDK2 as a selective target for CCNE1-amplified cell lines. We performed a high-throughput compound screen and identified a number of potential therapeutic combinations. We focused on dinaciclib and AKT inhibitors, and demonstrate selective and potent activity in CCNE1-amplified HGSC. We further show cooperation between CCNE1 and AKT, both in genomic data from TCGA and functionally in fallopian tube secretory cells. This study demonstrates approaches to target an important subset of solid cancers, and for the first time provides evidence to support the design of a rational clinical trial that targets CCNE1-amplified HGSC.

amplification represent a group with unmet clinical need, as they are unlikely to benefit from PARP inhibitors by virtue of the mutual exclusivity of CCNE1 amplification and BRCA1/2 mutation (7, 12), and are less likely to respond to platinum agents.

In recent preclinical studies, we have shown a dependency on CDK2 (13) and HR activity (12) in CCNE1-amplified cell lines. Although targeted agents have been effective in the clinical setting across many cancers, the emergence of acquired resistance is common (14). Indeed, we reported in vitro resistance to CDK2 inhibitors through selection of a polyploid population in the CCNE1-amplified cell line OVCAR3 (13). Rational drug combinations are a potential strategy to prevent resistance (15), and may also facilitate improvements in the therapeutic window by reducing the doses of drugs required to achieve efficacy, resulting in fewer side effects (16). We therefore used a high-throughput drug screen to identify drug combinations that synergize with the CDK2 inhibitor dinaciclib (17) to selectively target CCNE1-amplified HGSC, and to overcome resistance in a cell line that has acquired resistance to CDK inhibitors in vitro (13). We identified several synergistic combinations, including dinaciclib and AKT inhibitors, and found that this synergy extended more generally to CCNE1-amplified HGSC cell lines. Our results suggest targeting CDK2 and the AKT pathway may be an important approach to the clinical management of CCNE1-amplified HGSC.

Materials and Methods

Ethics statement

All animal experiments were approved by the Peter MacCallum Cancer Centre Animal Experimentation Ethics Committee and conducted in accordance with the National Health and Medical Research Council Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

Cell lines

Ovarian cancer cell lines were obtained from the National Cancer Institute Repository, actively passaged for less than 6 months, and authenticated using short-tandem repeat markers to confirm their identity against the Cancer Genome Project database (Wellcome Trust Sanger Institute, Cambridge, United Kingdom) before use in experiments. Cells were maintained at 37°C and 5% CO₂ (v/v), and cultured in RPMI1640 media containing 10% (v/v) FCS and 1% penicillin/streptomycin. Transfection and drug sensitivity assays were performed in the absence of antibiotics. Cell lines resistant to dinaciclib were generated utilizing methods as described previously (13). Briefly, OVCAR3 cells were plated in 6-well plates and treated with dinaciclib at the IC₅₀ dose for two 72-hour periods (media removed and fresh drug added). Surviving cells were allowed to repopulate for 96 hours and the process repeated once. Remaining cells were cultured in media or in the presence of drug, and regularly monitored for sensitivity to dinaciclib. Six independent cell lines were generated in this fashion, and designated OVCAR3-RD1 to -RD6.

Short hairpin–mediated CDK2 knockdown

Short hairpin–mediated knockdown of CDK2 was performed by cloning CDK2-specific shRNA into a lentiviral tetracycline-inducible expression vector containing the optimized miR-E backbone (18). The modified lentiviral vector pRRL-T3G-TurboGFP-miRE-PGK-mCherry-ires-rTA3 (also referred to as LT3GECIR) system includes a red (mCherry) fluorescent marker for transduction and a green (turboGFP) fluorescent marker for induction. Five CDK2-specific shRNA constructs were cloned into this system (see Supplementary Table S2 for sequences). For lentiviral production, HEK293T cells were transfected with plasmid DNA combined with the Lent-i-X packaging system (Clontech Laboratories). Transfection, production of lentiviral particles, and transduction of target cells was performed as described by the manufacturer’s protocol. Doxycycline was used to induce shRNA expression, and transfection efficiency was validated by flow cytometry (FACS), and knockdown of individual hairpins by RT-PCR and Western blot analysis. The most efficient shRNA construct was taken forward for in vitro and in vivo experiments.

For in vivo experiments, xenograft tumors from transduced cells were generated as described below. Once tumors reached 100 mm³, mice were randomized into two groups to receive either normal food and water or doxycycline food and water (2 mg/mL in 2% sucrose) as a means of reliable induction of shRNA expression. Tumors were subsequently monitored as described below.

Cyclin E1 and AKT overexpression in Fallopian tube secretory epithelial cells

The immortalized fallopian tube secretory epithelial cell (FTSEC) line FT282 was obtained from Ronny Drapkin (University of Pennsylvania, Philadelphia, PA; ref. 19). Derivative cell lines were generated using pMSCV-mCherry-(empty) and pMSCV-mCherry-CCNE1, encoding full-length CCNE1. Additional cell lines were generated with pMSCV-GFP-myr-AKT1, pMSCV-GFP-myr-AKT2, and pMSCV-GFP-myr-AKT3, encoding the three different isoforms of myr-AKT (20). Plasmids were validated by sequencing, and expression of CCNE1, AKT1, AKT2, and AKT3 was validated by quantitative real-time PCR and Western blotting. Primer sequences are listed in Supplementary Table S1.

High-throughput compound screen

The compound library consisted of 73 targeted agents, 71 epigenetic agents, 208 kinase inhibitors, and 3,707 known drugs
(21). All agents were dissolved in DMSO, and diluted to concentrations from 0.01 to 10 μmol/L. For targeted agents, epigenetic agents and kinase inhibitors, the primary screen was conducted using 11 concentrations; for the known drug library three concentrations were used. Compounds were dispensed into 384-well drug stock plates and stored at –20°C. Stock plates for dinaciclib at a fixed dose concentration (EC50) were prepared using a multichannel pipette before each assay.

Early passage cells were deposited into 384-well microtitre plates at 750–1,500 cells per well using a multidrop dispenser (Thermo Scientific) in 40 μL of media. Cells were allowed to adhere overnight. A MiniTrak IX (PerkinElmer Life Sciences) automated robotic platform was used to dispense compounds into assay plates. Compounds were added directly to assay plates using a 384, hydrophobic slotted pintool (VP Scientific) calibrated to dispense 0.1 μL of DMSO compound solution. DMSO (0.1%) was used as negative control. Cells were exposed to drug for 48 hours, and cell viability measured using the CellTiter-Glo Luminescent Assay (Promega) using the EnVision Multilabel Plate Reader (PerkinElmer). Average viability was normalized to DMSO control wells, and EC50 dose was approximated by fitting a four-parameter dose–response curve using XLfit (IDBS).

Xenograft studies
Estrogen pellets were implanted subcutaneously into 4- to 6-week-old female NOD/SCID mice to facilitate the growth of xenografted cells. The pellet was implanted 3 days before injection of cells. Cell lines were grown in vitro, washed twice with PBS, and resuspended in 50% Matrigel (BD Biosciences) in PBS. Mice were injected subcutaneously with 5 × 10^6 cells in 100 μL and monitored at least twice weekly. Tumor volume was calculated using the equation: volume = (width)^2 × length/2. When tumors reached 100 to 150 mm^3, mice were randomized into groups of five for treatment with vehicle alone or drug. Dinaciclib was prepared fresh before injection in 20% (w/v) hydroxypropyl-beta-cyclodextrin (Cyclodextrin Technologies Development, Inc.) and mice dosed twice weekly as a single agent via intraperitoneal injection. MK-2206 was reconstituted in 30% (w/v) Captisol (Ligand Technology) and dosed at 60 mg/kg three times per week as a single agent via oral gavage. For combination studies, MTDs of dinaciclib 20 mg/kg and MK-2206 60 mg/kg were dosed three times per week. All mice were monitored daily following drug dosing. Tumors were harvested at specific time points for biomarker analysis or at study endpoint, with half snap frozen in liquid nitrogen and half fixed in formalin and paraffin embedded for IHC. Percent age tumor growth inhibition (TGI) was calculated as 100 × (1 – ΔT/ΔC) where ΔC and ΔT were determined by subtracting the mean tumor volume (in the vehicle control and treated groups, respectively) on day 1 of treatment, from the mean tumor volume on each day of assessment. Statistical analyses were performed using GraphPad Prism Version 6.0 (GraphPad) with ANOVA followed by Dunnett post hoc test to compare the tumor growth between treatment groups.

CCNE1 and AKT status in primary ovarian tumor samples
Genomic alterations identified in CCNE1 and genes involved in the PI3K–AKT–mTOR pathway were obtained from The Cancer Genome Atlas (TCGA) cBioPortal (22, 23). All available data as of March 2015 were analyzed, comprising 316 primary ovarian serous cystadenocarcinoma samples (7).

shRNA screen data
Data from the Project Achilles was obtained to evaluate the interaction between CCNE1-amplified ovarian cancer cell lines and genes in the AKT pathway (24). Cell line copy number data were obtained from the Cancer Cell Line Encyclopedia (25). Only cell lines known to resemble HGSC according to their genomic characteristics (26) were used in the analysis (N = 14, see Table S3). Cell lines with a log2 copy number ratio > 0.3 over the CCNE1 locus were designated as amplified (n = 9) and cell lines with a log2 copy number ratio < 0 were designated as unamplified (n = 5). Cell lines with CCNE1 gene expression greater than the median + 1 SD (n = 9) were defined as CCNE1-high expression, whereas cell lines with CCNE1 gene expression less than median (n = 5) were defined as CCNE1-low expression.

Results

CCNE1-amplified HGSC cells are selectively sensitive to CDK2 knockdown
We previously demonstrated in a limited number of cell lines that CCNE1-amplified HGSC cell lines are selectively sensitive to CCNE1 and CDK2 knockdown mediated by siRNA (13). Following a recent analysis of ovarian cancer cell lines (26), we extended our analysis to a wider number of HGSC cell lines and confirmed consistent ampiclon-dependent sensitivity to siRNA-mediated CCNE1 and CDK2 knockdown (Fig. 1A and Supplementary Fig. S1A and S1B). The OVCA8 cell line has a low-level gain of CCNE1 and was not sensitive to CCNE1 or CDK2 knockdown (Fig. 1A). However, OVCA8 does not overexpress cyclin E1 at the mRNA or protein level (Supplementary Fig. S1B and S1C) compared with other cell lines such as OVCA4 which have similar CCNE1 copy number. These findings suggest a threshold of CCNE1/CDK2 dependency that may be relevant to patient selection in clinical trials targeting this oncogene in HGSC.

To validate the effect of CDK2 knockdown, we utilized a tetracycline-inducible shRNA targeting CDK2 (Fig. 1B). Consistent with the siRNA data, inhibition of CDK2 by shRNA resulted in reduced clonogenic survival, more evident in the CCNE1-amplified cell line, OVCA3 compared with the CCNE1-unamplified cell line CAOV3 (Fig. 1C). Knockdown of CDK2 was validated at the protein level (Supplementary Fig. S2A). Cell-cycle analysis demonstrated arrest in G1, seen only in the OVCA3 cell line (Fig. 1D). We did not observe significant levels of apoptosis following CDK2 knockdown, as assessed by percentage of Annexin V/propidium iodide cells measured by FACS (Supplementary Fig. S2B).

Cells transduced with CDK2-shRNA were grown as xenografts in NOD/SCID mice to examine the effects of CDK2 knockdown in vivo. Consistent with the in vitro data, attenuation of CDK2 expression in the OVCA3 xenograft model resulted in significant tumor growth arrest in the group receiving doxycycline in food and water compared with controls (Fig. 1E–F). Induction of shRNA by doxycycline was monitored by RT-PCR (Supplementary Fig. S2C). Reduced Rb1 phosphorylation was observed following CDK2 knockdown in OVCA3 tumors harvested at 7 days following induction (Fig. 1G), providing a biomarker of targeting cyclinE1/CDK2.

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Taken together, CCNE1-amplified HGSC appear selectively sensitive to siRNA- and shRNA-mediated knockdown of CDK2 both in vitro and in vivo. These findings support our previous studies and point to CDK2 as a potential therapeutic target in CCNE1-amplified HGSC.

CDK2 inhibitor dinaciclib delayed tumor growth in CCNE1-amplified HGSC xenografts

Consistent with siRNA data, we previously showed in a limited number of cell lines selective sensitivity of CCNE1-amplified cell lines to dinaciclib, a potent CDK2 inhibitor in advanced clinical development (13). However, in this study, when tested across a broader panel of HGSC cell lines, there did not appear to be a clear ampiclon-dependent sensitivity (Fig. 2A), in contrast with the siRNA and shRNA data. Furthermore, activity in vitro was also seen in a xenograft model developed from a CCNE1-unamplified cell line, CAOV3 (Fig. 2A–D). The difference in ampiclon-dependent sensitivity between gene suppression and pharmacologic inhibition may be due to the broad activity of dinaciclib, which, in addition to inhibiting CDK2, is also active against CDK1, 5, 9, and 12 (17, 27).

In addition to CDK2 inhibitors, we previously identified use of bortezomib, a proteasome inhibitor, as a potential therapeutic strategy for CCNE1-amplified HGSC (12). Although we did not observe ampiclon-dependent sensitivity to dinaciclib, we investigated the interaction between dinaciclib and bortezomib to see whether the two drugs would be synergistic in combination. Using the Chou–Talalay methodology for drug combination studies (28), we did not observe a synergistic interaction with dinaciclib and bortezomib (Fig. 2E and F) in a panel of CCNE1-amplified and CCNE1-unamplified HGSC cell lines. Given this lack of synergism, we sought to identify selective synergetic drug combinations by adopting an unbiased high-throughput screening approach.

A high-throughput compound screen identifies synergetic drug combinations

We performed a high-throughput compound screen to identify combinations that would be synergistic in CCNE1-amplified cells, as well as combinations that would be selective in a CDK inhibitor–resistant cell line OVCAR3-R1-533533 (13). In the primary screen, 4,059 compounds (including duplicates) were combined with a fixed dose of dinaciclib as described in Materials and Methods. Dose–response curves were generated and manually curated, and compounds where a curve could not be fitted were excluded from the analysis. A full list of EC_{50} values for each cell line and compound is given in Supplementary Tables S4 and S5. EC_{50} values from the primary screen were used to make two pair-wise comparisons (Fig. 2G and H): (i) dinaciclib plus library compound comparing OVCAR3 (CCNE1-amplified) versus SKOV3 (CCNE1-unamplified) and (ii) dinaciclib plus library compound comparing OVCAR3 (parental) and OVCAR3-R1 (CDK inhibitor resistant). At the time of undertaking the screen, SKOV3 was a commonly used ovarian cancer cell line; however, recent studies have demonstrated that SKOV3 is unlikely to resemble HGSC (26). Therefore, any potential hits identified in the screen were subsequently validated using only HGSC cell lines.

Library compounds where the ratio of EC_{50} was less than 0.5 were selected as hits for a secondary screen involving a total of 64 compounds (Supplementary Table S6 and S7). Compounds that appeared to have an additive effect with dinaciclib were selected as hits from the secondary screen and carried forward for further testing.

The final part of the screen involved assessing the level of synergy between the library compound hits and dinaciclib involving an 11-point titration of each compound. Using the Chou–Talalay methodology of constant-ratio drug combinations, a series of combination indexes were generated to identify synergetic interactions.

In the OVCAR3 parental cell line, there were no synergistic combinations identified between dinaciclib and the library compounds (Supplementary Table S8). In the OVCAR3-R1 cell line, there were a number of synergistic interactions identified (Supplementary Table S8). Nonselective BH3-mimetic agents ABT-263 and ABT-737 were synergistic in combination with dinaciclib, suggestive of a class effect. This was validated further in an independently derived dinaciclib-resistant cell line, OVCAR3-RD6 (Fig. 3A–B and Supplementary Fig. S4A–S4C). There was no synergistic interaction noted in the combination between dinaciclib and ABT-199 (Fig. 3C), a selective Bcl-2 antagonist. The combination of dinaciclib and ABT-737 resulted in a dose-dependent increase in apoptosis, observed only in CDK inhibitor–resistant cell lines as demonstrated by increase in PARP cleavage products on Western blot analysis (Fig. 3D). Mcl-1 protein expression was not observed in the OVCAR3-RD6 cell line resistant to dinaciclib (Fig. 3D). Real-time PCR demonstrated upregulation of antiapoptotic genes in the dinaciclib and
Figure 2.
CDK inhibitor dinaciclib results in modest tumor growth inhibition in vivo but is not synergistic in combination with bortezomib in vitro. A, Mean IC_{50} values for a panel of HGSC cell lines treated with dinaciclib generated from dose-response curves following standard MTS cell proliferation assays. Error bars, SEM, n = 3 experiments. B, In vivo effects of dinaciclib. Immunocompromised mice bearing OVCAR3 (CCNE1-amplified) or CAOV3 (CCNE1-unamplified) tumor xenografts were treated with vehicle or drug as described in Materials and Methods. Plots represent mean tumor volume change from baseline ± SEM, n = 5 mice per group. C, the percentage tumor growth inhibition following 21 days of treatment with vehicle or dinaciclib. Bars represent mean ± SEM, n = 5 mice per group. Statistical analysis performed with ANOVA followed by Dunnett post hoc test to compare the percentage tumor growth inhibition between the treatment groups. **, P < 0.01. D, Immunohistochemical analysis of Ki67 expression in OVCAR3 and CAOV3 tumor xenograft harvested 24 hours after dose of vehicle or dinaciclib. E, Formal assessment of synergy between dinaciclib and bortezomib using Chou-Talalay Isobologram analysis. Figures are generated with CalcuSyn 2.0. Data are normalized, with connecting line at X and Y corresponding to combination index = 1, representing line of additivity. Data points above the line are antagonistic, along or near the line are additive and points below the line are synergistic. F, Combination indexes for a panel of HGSC cell lines tested against dinaciclib in combination with bortezomib. Values represent mean ± SEM, n = 3. G–H, Scatter plots showing IC_{50} values for library compounds in combination with dinaciclib from primary screen for the comparison between CCNE1-amplified and unamplified (G) and resistant versus parental (H). Data points in red represent compounds taken forward for secondary screen.
Figure 3.
Dinaciclib in combination with nonselective BH3 mimetics are synergistic in CDK inhibitor–resistant cell lines. Combination indexes for parental and CDK inhibitor–resistant cell lines tested against dinaciclib in combination with ABT-737 (A), ABT-263 (B), ABT-199 (C). Values represent mean ± SEM, n = 3. D, Western blot analysis demonstrating protein expression of Bcl-XL, Mcl-1, and PARP cleavage products in OVCAR3 parental and CDK inhibitor–resistant cell lines after treatment with dinaciclib and ABT-737. E, Expression of antiapoptotic proteins as assessed by quantitative real-time PCR. R-lines signify cell lines resistant to PHA53533. RD lines signify cell lines resistant to dinaciclib. Bars represent mean ± SEM, n = 3.
Figure 4.
Dinaciclib in combination with two AKT inhibitors are synergistic in vitro and in vivo models of CCNE1-amplified HGSC. Combination indexes for a panel of HGSC cell lines tested against dinaciclib in combination with MK-2206 (A) and GSK2110183 (B). Values represent mean ± SEM. (Continued on the following page.)
Dinaciclib and MK-2206 are selectively synergistic in ccne1-amplified cell lines in vitro

The in vivo effect of dinaciclib and MK-2206 was assessed using xenograft models from ccne1-amplified and unamplified cell lines, OVCAR3 and CAOV3, respectively. The combination was significantly more effective than each single agent alone in the CCNE1-amplified model (Fig. 4D and E), whereas there was no statistically significant effect of the combination compared to single-agent treatment in the CCNE1-unamplified model. After a treatment period of three weeks with dinaciclib and MK-2206, xenograft tumors began regrowing within 10 days of treatment cessation. Rechallenge with the same drug combination resulted in significant tumor regression (Supplementary Fig. S4F), indicating continued sensitivity to the combination. Consistent with this effect on tumor growth, treatment with dinaciclib and MK-2206 resulted in inhibition of cell proliferation and induction of apoptosis, as assessed by ki67 and cleaved caspase-3 IHC on tumors harvested at 24 hours (Fig. 4F and G). Taken together, the high-throughput screen identified a novel combination of dinaciclib and MK-2206 that appeared to be selectively synergistic in ccne1-amplified HGSC cell lines both in vitro and in vivo.

Cyclin E1 and AKT overexpression cooperates to promote uncontrolled growth in FTSECs

Previously, Karst and colleagues demonstrated that cyclin E1 overexpression combined with TP53 deletion in FTSECs results in increased proliferation, colony-forming ability, and colony formation in soft agar (19). However, cyclin E1 overexpression alone did not result in complete transformation, suggesting that additional events are required.

We examined the interaction between cyclin E1 and AKT overexpression in FTSECs by overexpressing the myristoylated, active forms of AKT1, AKT2, and AKT3 (20). Expression of each AKT isoform and cyclin E1 was validated with Western blot analysis (Fig. 6A) and RT-PCR (Supplementary Fig. S6A). Overexpression of AKT isoforms led to increased expression of AKT downstream targets (Supplementary Fig. S6B). AKT2 and cyclin E1 overexpression alone or in combination showed a trend toward increased proliferation compared with empty vector alone (Fig. 6B); however, AKT2 or AKT3 overexpression in combination with cyclin E1 showed a trend toward enhanced clonogenic colony formation in comparison with overexpression of cyclin E1 alone (Fig. 6C).

There was a significant increase in soft agar colony formation with the overexpression of AKT2 or AKT3 in combination with cyclin E1.
cyclin E1 compared with overexpression of cyclin E1 alone (Fig. 6D). These findings support an interaction between cyclin E1 and AKT pathway to promote uncontrolled growth in FTSECs, and may explain synergism observed between dinaciclib and MK-2206 in CCNE1-amplified HGSC.

**Discussion**

HGSC patients with CCNE1 amplification have a clear unmet need in terms of effective therapies. In this study, we validate CDK2 as a selective target in CCNE1-amplified HGSC using shRNA-mediated gene suppression in vitro and in vivo. However, we did not observe similar amplicon-dependent specificity to dinaciclib, a small-molecule inhibitor targeting CDKs. This may be due to the nonspecificity of inhibitors such as dinaciclib or a role for kinase-independent activities of CCNE1 in amplified HGSC (30). Our findings highlight the potential differences between inhibition of kinase activity and complete suppression of CCNE1 or CDK2 gene expression.

In addition to CDK2, dinaciclib targets CDK1, 5, 9, and 12 (17, 27). CDK9 phosphorylates the carboxyl-terminal repeat domains of RNA polymerase II, and inhibition of CDK9 by dinaciclib results in rapid downregulation of mRNA transcripts and proteins with short half-lives such as the antiapoptotic BCL2 family member, Mcl1 (17). Preclinical studies have indicated dinaciclib-mediated targeting of Mcl-1 may be an effective therapeutic approach in a number of different cancers (17). Inhibition of CDK2 kinase activity may also differ significantly from complete suppression of gene expression, resulting in varying downstream and compensatory effects (31, 32). Studies with knockout experiments indicate that CDK2 functions appear redundant with CDK1, although in our studies, we did not observe upregulation of CDK1 expression following CDK2 knockdown in vitro or in vivo (data not shown).

Although we observed a difference in the amplicon-dependent sensitivity of CDK2 gene suppression compared with pharmacologic inhibition, dinaciclib remains a potent CDK2 inhibitor with single-agent activity in CCNE1-amplified HGSC cell lines and is one of the most clinically advanced CDK2 inhibitors (33). Therefore, to more effectively target CCNE1-amplified HGSCs, we performed a combinatorial drug screen to identify compounds that would synergize with dinaciclib. We also sought to identify compounds that may potentially overcome resistance to dinaciclib, a common occurrence in the clinical use of targeted small-molecule inhibitors, by testing a cell line that was resistant to CDK inhibitors. Dinaciclib in combination with MK-2206, an AKT inhibitor, was identified as a synergistic combination in targeting CDK inhibitor–resistant cell lines. This supported our previous work that identified increased AKT1 copy number and upregulation of genes in the AKT pathway as a potential mechanism of resistance to CDK2 inhibitors (13). In validating this finding, we observed selective, potent synergism between dinaciclib and MK-2206 in vitro and in vivo models of CCNE1-amplified HGSCs, including parental OVCAR3 cells. This interaction was not
Figure 6.
Cyclin E1 and AKT overexpression cooperates to promote uncontrolled growth in FTSECs. A, Western blot analysis of fallopian tube secretory cells transduced with cyclin E1, empty vector, and AKT1, AKT2, and AKT3 overexpression constructs. Blots are representative of three independently performed experiments. B, Proliferation assay of fallopian tube secretory cells (FT282) transduced with empty vector (EV), cyclin E1 (CCNE1), AKT2, and both cyclin E1 and AKT2 (CCNE1 + AKT2). Plots represent mean of three independently performed experiments, error bars represent SEM. C, Clonogenic survival assay of FT282 cells transduced as labeled. Images (left) show cells fixed and stained with crystal violet. Bar chart represents mean of three independently performed experiments, error bars represent SEM. Statistical significance (t test) calculated by comparison with FT282 cells transduced with cyclin E1 (FT282-CCNE1). D, Anchorage-independent assay of FT282 cells transduced as labeled. Images (left) represent cells fixed with 2% paraformaldehyde and captured using an Olympus IX81 live cell imager. Bar chart represents mean of three independently performed experiments, error bars represent SEM. Statistical significance (t test) calculated by comparison with FT282 cells transduced with cyclin E1 (FT282-CCNE1); *, P < 0.05; **, P < 0.01.
Initially observed in the primary high-throughput screen. However, the use of SKOV3 cell line as a comparator in the screen may be a potential confounder, as the selection of compounds as hits from the primary screen was based on a difference in the EC₅₀ values between the two cell lines tested, OVCAR3 and SKOV3. Recently, multiple studies characterizing the genomic profile of commercially available ovarian cancer cell lines have shown that many of these cell lines, including SKOV3, may not accurately resemble HGSC (26, 34–36).

Synergism between dinaciclib and MK-2206, as well as another AKT-specific inhibitor GSK2110183, but an absence of a synergistic combination with other inhibitors of the PI3K–AKT–mTOR pathway suggests that the interaction with CCNE1 may be specific to AKT. Analysis of genomic data from patients demonstrated a significant cooccurrence of CCNE1 and AKT2 amplification, which may in part be explained by colocalization on chromosome 19q. However, FUOV1, which has CCNE1 amplification without AKT2 amplification (25), was equally sensitive to the combination of dinaciclib and AKT inhibitors. Coexpression of AKT2 or AKT3 with cyclin E1 in a TP53-mutant FTSEC cell line resulted in increased proliferation and anchorage-independent growth. Analysis of data from Project Achilles indicates that HGSC cell lines that have CCNE1 amplification or overexpression are dependent on multiple genes within the AKT pathway. We previously performed a pathway analysis of genes coexpressed with CCNE1 amplification and observed an enrichment of genes involved in AKT signaling (12). Collectively, these data suggest a specific dependency of CCNE1-amplified tumors for AKT activity.

Dinaciclib and MK-2206 have previously been shown to be active against pancreatic adenocarcinoma (37). In KRAS-mutant pancreatic cancer patient–derived xenografts, Hu and colleagues (37) demonstrated efficacy of dinaciclib combined with MK-2206. They proposed that sensitivity was due to the effect of dinaciclib on CDK5, and in turn, inhibition of RAL pathway. On the basis of these results, a phase 1 clinical trial (NIH Trial NCT01783171) of dinaciclib and MK-2206 has been initiated in patients with advanced pancreatic cancer. While this trial will provide safety and recommended dosing of the combination, patients are not preselected on the basis of tumoral CCNE1 amplification or overexpression. The mechanism of interaction and biomarkers that predict response are likely to be different in pancreatic cancer compared with HGSC.

Other combinations were also identified from the high-throughput screen. In particular, nonselective BH3-mimetic compounds ABT-737 and ABT-263 were synergistic in combination with dinaciclib in CDK inhibitor–resistant cell lines. There was no synergistic interaction between dinaciclib and the Bel-2–specific antagonist, ABT-199, indicating that the targeting of multiple antiproliferative proteins is potentially required to overcome resistance to CDK2 inhibitors. This observation is supported by upregulation of multiple genes in this pathway including BCL-2, BCL-XL, and BCL-W in resistant cell lines. However, the use of ABT-737 or ABT-263 in combination with dinaciclib in vivo is hindered by significant toxicities, particularly hematologic (Joel Levenson, personal communication), and are therefore unlikely to have clinical utility.

Biomarker-driven trials in HGSC are needed to improve clinical outcomes. HGSC patients with CCNE1 amplification are a subset that requires different treatment approaches, given that they have HR-proficient tumors, and as such, are likely to have poor responses to platinum-based chemotherapy and PARP inhibitors. However, targeted therapies when used alone may not be sufficient to induce selective, cytotoxic effects, and often result in the development of resistance. Combination therapies may potentially be a strategy to overcome these limitations. High-throughput drug screening is an unbiased approach to identify novel therapeutics, and we have identified dinaciclib and MK-2206 as a combination that may prove to selectively target patients with CCNE1-amplified HGSC. Further work incorporating additional clinically relevant models and novel combinations will inform the design of rational clinical trials targeting CCNE1-amplified HGSC.

Disclosure of Potential Conflicts of Interest

There were no potential conflicts of interest.

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